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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12N 15/82, A01H 5/00 — A1

(11) International Publication Number: WO 94/29464

(43) International Publication Date: 22 December 1994 (22.12.94)

(21) International Application Number: PCT/EP94/01817

(22) International Filing Date: 3 June 1994 (03.06.94)

(30) Priority Data:
9311593.9 4 June 1993 (04.06.93) GB

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(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: VIRUS RESISTANT PLANTS

(57) Abstract

DNA constructs encoding an RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by a virus when invading a plant such that either an eliciting element or a plus sense RNA is produced as a consequence of the interaction with the RNA dependent RNA polymerase encoded by the said invading virus, whereby any produced plus sense RNA molecule is capable of encoding for an eliciting element, plants containing such constructs and processes for obtaining such plants.

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VIRUS RESISTANT PLANTS

BACKGROUND

The present invention relates to pathogen resistant plants and in particular to pathogen resistant plants wherein pathogen resistance is triggered in response to invading pathogens such as viruses, DNA constructs for use in such plants and methods of introducing virus induced resistance into plants.

Viral infections in plants are frequently responsible for detrimental effects in growth, undesirable morphological changes, decreased yield and the like. Such infections often result in a higher susceptibility to infection in infected plants to other plant pathogens and plant pests.

Virus particles generally comprise a relatively small amount of genetic material (single or double stranded RNA or DNA) protected by a protein or proteins which in some viral types can also be surrounded with host-derived lipid membranes, yielding infectious particles. Viruses are dependent on host cells for multiplication and may therefore be regarded as intracellular parasites.

Plants have evolved a number of defensive mechanisms to limit the effects of viral infection. For example, so-called horizontal or partial resistances which are polygenic in nature and so-called vertical resistances which are monogenic in nature.

Horizontal resistance is difficult to introduce successfully into plants in breeding programs, however, vertical resistance can be bred into plants relatively easily within plant breeding programs. Genes coding for virus resistance can act constitutively in a passive sense, ie without a requirement for inducing gene expression. Constitutively expressed virus resistances include as modes of action non-host resistances,

tolerance ie inhibition of disease establishment, immunity ie inhibition of transport or the presence of antiviral agents and the like. Alternatively, genes coding for virus resistance in plants can be actively switched on by way of inducing expression of a gene or genes encoding for a viral resistance. An example of such a system includes the hypersensitive response.

So-called hypersensitive responses (HSR) in plants have been reported and are generally characterized by death of plant cells in the vicinity of the penetrating pathogen shortly after infection. Movement of the pathogen through infected or invaded cells is restricted or blocked due to necrosis of the invaded cell and/or cells in the environs of the invaded cell(s). In addition, HSR involves a cascade of additional or secondary defense responses and the accumulation of certain proteins and secondary metabolites, leading to a general increased level of resistance to attack by pathogens. HSR reactions to invading organisms are generally thought to involve a resistance gene product in the plant cell which recognizes and interacts with an elicitor element, ie the product of an avirulence gene of a pathogen. Elicitor element recognition in the cells of a resistant plant triggers an HSR reaction which in its turn restricts the pathogen infection to a single cell or cells, or at most to a few plant cells in the immediate vicinity thereof.

An example of HSR-mediated resistance to virus infection is that of tobacco plants harbouring the N' resistance gene to tobamoviruses such as TMV and ToMV, which contain the coat protein avirulence gene. Thus far, more than twenty single dominant HSR-type resistance genes have been identified, and are present in many agronomically important crops including tobacco, tomato, potato, pepper, lettuce, and the like.

Despite the apparent abundance of resistance sources to certain viruses, many crops still lack effective resistance genes to

important viral pathogens [Fraser, R.S.S. (1992). Euphytica 63:175]. Searching of wild type germplasm collections has identified only a few suitable sources of viral resistance capable of being introduced successfully into agronomically important crops. An example is the absence of vertical resistance genes to cucumber mosaic virus (CMV) in many agronomically important crop types including but not limited to tomato, pepper, cucumber, melon, lettuce and the like.

Plant breeders continuously try to develop varieties of crop plant species tolerant to or resistant to specific virus strains. In the past , virus resistance conferring genes have been transferred from wild types related to commercial plants into commercial varieties through breeding. The transfer of an existing resistance in the wild from the wild type gene pool to a cultivar is a tedious process in which the resistance conferring gene(s) must first be identified in a source (donor) plant species and then combined into the gene pool of a commercial variety. Resistance or tolerance generated in this way is typically active only against one or at best a few strains of the virus in question. A further disadvantage is that the breeding programme generally takes a long time, measured in years, in getting to agronomically useful plants.

In an alternative, a system referred to as "cross-protection" has been employed. Cross-protection is a phenomenon in which infection of a plant with one strain of a virus protects that plant against superinfection with a second related virus strain. The cross-protection method preferentially involves the use of avirulent virus strains to infect plants, which act to inhibit a secondary infection with a virulent strain of the same virus. However, the use of a natural cross-protection system can have several disadvantages. The method is very labour intensive because it requires inoculation of each individual plant crop, and carries the risk that an avirulent strain may mutate to a virulent strain, thus becoming a causal agent for crop disease in itself. A further possible hazard is

that an avirulent virus strain in one plant species can act as a virulent strain in another plant species.

Genetically engineered cross-protection is a form of virus resistance which phenotypically resembles natural cross-protection, but is achieved through the expression of genetic information of a viral coat protein from the genome of a genetically manipulated plant. It is known that expression of the tobacco mosaic virus strain U1 (TMV-U1) coat protein gene from the genome of a transgenic plant can result in a delay of symptom development after infection with any TMV strain. Similarly, coat protein-mediated protection has also been obtained for alfalfa mosaic virus (AMV), potato virus X (PVX) and cucumber mosaic virus (CMV). For some plant viruses, eg luteoviruses, it is difficult to obtain detectable amounts of the corresponding coat protein in a transgenic plant, and consequently, virus resistance is generally lowered. Furthermore, any alleged degree of protection requires that the plant produces coat protein continually and thus imposes an energy burden on the plant. As a result of such limitations the commercial value of such technology remains unclear.

A further example of genetically engineered virus resistance includes the introduction of plant viral satellite RNA wherein expression of incorporated genetic material modifies the plant virus or its effects.

An object of the present invention is to provide an alternative more reliable engineered virus resistance strategy in plants to those engineered resistances known in the art, based on direct pathogen induced expression of molecules in target tissues of a plant before the invading pathogen can establish itself in the host plant.

Another object of the invention is to combine genetic engineering plant transformation technology with naturally existing plant viral defense mechanisms in plant tissue.

Detailed Description

According to the present invention there is provided a plant virus DNA construct capable of encoding directly or indirectly for a minus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by an invading virus such that at least one eliciting element is produced as a consequence of the interaction with the RNA dependent RNA polymerase encoded by the said invading virus.

In another embodiment of the invention there is provided a plant virus recombinant DNA construct capable of encoding for a plus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by an invading virus producing as a result of such interaction, a plus sense RNA molecule which is capable of encoding for at least one eliciting element capable of eliciting a natural plant defense in the plant on invasion of the plant by the said invading virus.

The plant virus DNA construct can be derived from any virus source capable of attacking a plant, however it is preferred that the plant virus DNA is derived from any virus source which is known to attack, is suspected of attacking or is capable of attacking an agronomically attractive plant type. It can be a natural plant virus DNA suitably modified for expression or it may be derived synthetically. The plant virus DNA should be capable of encoding for transcription into an RNA sequence complementary (ie minus sense) to a viral RNA (ie plus sense) in plant cells. In addition, the plant virus DNA should contain a portion or segment thereof which when transcribed to yield minus sense RNA and further transcribed to plus sense RNA, upon translation of the plus sense RNA, is capable of giving rise to at least one eliciting element or part thereof sufficient to elicit a natural plant defense mechanism against an invading virus. Suitable plant virus DNA or RNA sources are those derived from plant viruses capable of invading plant

types such as tomatoes, peppers, melons, lettuces, cauliflowers, broccolis, cabbages, brussels sprouts, sugar beet, corn (maize), sweetcorn, onions, carrots, leeks, cucumbers, tobacco and the like. Also included as plant virus DNA or RNA sources are those derived from plant viruses capable of invading plant types from ornamental crops such as Impatiens, begonia, petunia, pelargoniums (geraniums, viola, cyclamen, verbena, vinca, tagetes, primula, saintpaulia and the like.

A minus sense RNA molecule is one which contains at least a cistron or part thereof corresponding to at least a portion of the said plant virus DNA and is capable of giving rise to a plus sense RNA molecule transcribable from the said minus sense RNA molecule which is capable of coding for and giving rise to at least one eliciting element or part thereof in plant cells. The minus sense RNA may be directly transcribable from the said plant virus DNA or it may be transcribable from a plus sense RNA derived from the said DNA. As such, the minus sense RNA transcribable from a plus sense RNA is referred to, for the purposes of the present invention, as being indirectly transcribable from the said DNA. The orientation or polarity of the cistron or cistrons or parts thereof located on the minus sense RNA molecule can be such that the eliciting element may not be directly coded for after transcription from the plant virus DNA construct. The genetic code of the cistron or cistrons or parts thereof is located on the complementary strand to the minus sense RNA molecule ie the plus sense RNA. The cistron coding for an eliciting element becomes available for translation when the minus sense viral RNA sequence is replicated by an RNA dependent RNA polymerase encoded for by an invading virus to yield a plus sense RNA molecule.

Minus sense RNA herein also includes those RNA molecules which can be described as having ambisense characteristics, such as RNA molecules from tospoviruses and the like. In such cases, the minus sense RNA contains at least a cistron corresponding

to a portion of the said plant virus DNA and is capable of giving rise to a plus sense RNA transcribable from the said minus sense RNA which is capable of coding for and giving rise to at least one eliciting element or part thereof in plant cells.

A plus sense viral RNA molecule is one which is capable of directly or indirectly encoding at least one eliciting element or part thereof capable of being expressed in, and having a natural or engineered plant defense eliciting activity in plant cells. A plus sense RNA molecule is also one which is complementary to a viral minus sense RNA and is capable of giving rise directly or indirectly to at least one elicitor element upon translation in plant cells. Thus, a viral sense RNA molecule can be viewed as a complementary RNA molecule to a minus sense RNA molecule.

Plus sense RNA herein also includes those RNA molecules which can be described as having ambisense characteristics. In such cases, the plus sense RNA contains at least a cistron corresponding to a portion of the said plant virus DNA and is capable of directly coding for and giving rise to at least one eliciting element or part thereof in plant cells.

The amount of eliciting element which is expressed in the plant cell must be sufficient to elicit at least a cellular plant defense response against an invading virus resulting in a natural or engineered plant reaction effective in blocking or restricting further viral action. Thus, plus sense RNA molecules whether they be the complement of a minus sense RNA or an ambisense RNA must be capable of giving rise to elicitor elements which are capable of triggering or eliciting a natural or engineered plant defence response, whether that be through direct translation or through interaction with a viral RNA dependent RNA polymerase (eg via a generated subgenomic RNA). There can be one or more eliciting elements ultimately encoded by the plus sense RNA depending on the type of plant defense

response/plant defence responses being elicited. The viral plus sense RNA sequence is preferably one wherein at least a viral cistron has been replaced by at least a cistron coding for an eliciting element capable of being expressed in plant cells, and having a natural or engineered plant defense eliciting activity in plant tissue.

The eliciting element can be any element translatable from a plus sense RNA cistron derivable from a plant virus DNA as hereinbefore described, and can be a protein, polypeptide, or peptide or fragments thereof. Examples of preferred eliciting elements include the so-called elicitor proteins and/or cell inhibitory proteins.

An elicitor protein is one which if present in plant tissue, is capable of eliciting, triggering, or inducing a hypersensitive response (HSR), that is a natural plant defense mechanism against invading pathogens such as viruses. Elicitor proteins can be of plant virus origin, such as coat proteins, proteins involved in cell-to-cell movement, helicases, RNA-dependent RNA polymerases and the like. In addition, elicitor proteins can originate from or be derived from other plant pathogens such as bacteria, fungi, nematodes and the like.

A cell inhibitory protein is a protein which if present in plant tissue, has a detrimental effect on the plant cell, leading to inhibition of cell growth eg cell division, and/or cell death. Cell inhibitory agents include but are not restricted to ribonucleases, proteinases, ribosomal inhibitory proteins, cell wall degrading proteins and the like.

The minus and plus sense RNA molecules can be viewed as plant virus RNAs since they are derived from a plant DNA construct as hereinbefore described and comprise the genome or a segment of the genome of a plant virus. In such plant virus RNAs, selected nucleotide fragments can be replaced by others or can be deleted. Replacement and/or deletion of nucleotides or

segments comprised of nucleotides should be such so as not to interfere with the capability of the RNA molecule to multiply or replicate in virus-infected plant cells. Also, replacement and/or deletion of nucleotides, codons or segments comprised of nucleotides should be such so as not to interfere with the ability of the RNA dependent RNA polymerase of the invading virus to recognise and act upon an RNA molecule (in plus or minus sense orientation), and thereby initiating the sequence of events as described herein leading to the production of an effective amount of an eliciting element capable of eliciting a natural or engineered plant defense response. Examples of suitable plant virus RNA molecules include, but are not limited to genomic RNA molecules or segments thereof selected from the group comprising potyviruses, potexviruses, tobamoviruses, luteoviruses or genomic RNA or segments thereof of cucumoviruses, bromoviruses, tospoviruses and the like.

The plant virus DNA is under expression control of a promoter capable of functioning in plants and includes a terminator capable of functioning in plants.

A promoter is the nucleotide sequence upstream from the transcriptional initiation site and which contains all the regulatory regions required for transcription. Examples of promoters suitable for use in DNA constructs of the present invention include viral, fungal, bacterial, animal and plant-derived promoters capable of functioning in plant cells. A preferred promoter should express the DNA constitutively, that is in all living tissues of the plant. It will be appreciated that the promoter employed should give rise to the expression of the viral plant DNA at a rate sufficient to produce the amount of RNA capable of encoding for at least an elicitor element capable of eliciting a natural plant defense in a transformed plant on invasion of the plant by a virus. The required amount of RNA to be transcribed may vary with the type of plant. Examples of suitable promoters include the cauliflower mosaic virus 35S (CaMV 35S) and 19S (CaMV 19S)

promoters, the nopaline synthase and octopine synthase promoters, the heat shock 80 (hsp80) promoter and the like.

A terminator is contemplated as (A) a DNA sequence downstream of the viral DNA, coding for transcription into an RNA sequence which is capable of autocatalytical, self cleavage, to release the terminator sequences from the recombinant viral RNA sequence, followed by (B) a DNA sequence at the end of a transcriptional unit which signals termination of transcription. These elements are 3'-non-translated sequences containing polyadenylation signals, which act to cause the addition of poly adenylate sequences to the 3' end of primary transcripts. Examples of sequences mentioned under (A) include self-cleaving RNA molecules or ribozymes such as ribonuclease P, *Tetrahymena* L-19 intervening sequence, hammerhead ribozymes, *Hepatitis* delta virus RNA, *Neurospora* mitochondrial VS RNA and the like [Symons, R.H. (1992). Ann. Rev. Biochem. 61:641]. Sequences mentioned under (B) may be isolated from funghi, bacteria, animals and/or plants. Examples, particularly suitable for use in the DNA constructs of the invention include the nopaline synthase polyadenylation signal of *Agrobacterium tumefaciens*, the 35S polyadenylation signal of CaMV and the zein polyadenylation signal from *Zea mays*.

A DNA or RNA sequence is complementary to another DNA or RNA sequence if it is able to form a hydrogen-bonded complex with it, according to rules of base pairing under appropriate hybridization conditions. For the purposes of the present invention appropriate hybridization conditions may include but are not limited to, for example, an incubation for about 16 hours at 42°C, in a buffer system comprising 5 x standard saline citrate (SSC), 0.5% sodium dodecylsulphate (SDS), 5 x Denhardt's solution, 50% formamide and 100 µg/ml carrier DNA or RNA (hereinafter the buffer system), followed by washing 3x in buffer comprising 1 x SSC and 0.1% SDS at 65°C for approximately an hour each time. Thus the hybridisation signal obtained for an RNA or DNA molecule, for example an

autoradiogram reading, should be sufficiently clear to the man skilled in the art so as to suggest that an RNA or DNA molecule obtained could usefully be employed in the construction of plant virus DNA constructs suitable for use in the invention. Naturally, such an RNA or DNA molecule should be capable of the requisite activity as described herein. Thus replacement and/or deletion of nucleotides, codons or segments comprised of nucleotides should be such so as not to interfere with the ability of a DNA construct of the invention to code for a minus sense RNA molecule as herein described which is capable of being recognised by and of interaction with an RNA dependent RNA polymerase of an invading virus and thereby initiating the sequence of events as described herein leading to the production of an effective amount of an eliciting element capable of eliciting a natural or engineered plant defense response.

Suitable hybridization conditions employed in the present invention can involve incubation in a buffer system for about 16 hours at 49°C and washing 3x in a buffer comprising 0.1 x SSC and 0.1% SDS at 55°C for about an hour each time. More preferably, hybridization conditions can involve incubation in a buffer system for about 16 hours at 55°C and washing 3x in a buffer comprising 0.1 x SSC and 0.1% SDS at 65°C for approximately an hour each time. Naturally, any RNA or DNA molecule subjected to such hybridisation conditions should be capable of the requisite activity as described herein.

The invention also provides a vector capable of introducing the DNA construct of the invention into plants and methods of producing such vectors. The term vector employed herein refers to a vehicle by means of which DNA molecules or fragments thereof can be incorporated into a host organism. Suitable vehicles include plasmids, naked DNA introduced using micro-injection, particle guns, and the like [Offringa (1992). PhD thesis, State University Leiden, The Netherlands, Ch1:pages 7-28].

The term plants as used herein is used in a wide sense and refers to differentiated plants as well as undifferentiated plant material such as protoplasts, plant cells, seeds, plantlets and the like which under appropriate conditions can develop into mature plants, the progeny thereof and parts thereof such as cuttings and fruits of such plants.

The invention further provides plants comprising in their genome a DNA construct of the invention, and methods of producing such plants.

The plants according to the invention have reduced susceptibility to diseases caused by the respective viruses and do not have the disadvantages and limitations of plants obtained by classical methods and genetic engineering methods as discussed herein.

The invention is illustrated by the following non-limiting examples and accompanying figures.

Figure 1: Schematic representation of the interaction of pathogen and plant encoded proteins leading to induction of an HSR response.

Figure 2: Schematic representation of CMV resistant tobacco or tomato plants, obtained by expression of a minus-sense CMV RNA 3 molecule in which the MP gene is replaced by a gene coding for an elicitor (ToMV CP or P30) or a cell inhibitory protein (RNase T1).

Figure 3: Schematic representation of CMV resistant tobacco or tomato plants obtained by expression of a plus-sense CMV RNA 3 molecule in which the CP gene is replaced by a gene coding for an elicitor (ToMV CP or P30) or a cell inhibitory protein (RNase T1).

Sequence ID 1: Chimaeric cucumber mosaic virus RNA 3.

Sequence ID 2: Coat protein of ToMV (corresponding to nucleotides from positions 123-600 of Seq. ID. No.1).

Sequence ID 3: Coat protein of cucumber mosaic virus corresponding to nucleotide positions from 897-1550 of Seq. ID. No 1.

Sequence ID 4: Chimaeric cucumber mosaic virus RNA 3.

Sequence ID 5: RNAse T1 corresponding to positions 123-437 of Seq. Id No. 4.

Sequence ID 6: Chimaeric cucumber mosaic virus RNA 3, coding for P30 of ToMV.

Sequence ID 7: P30 of ToMV corresponding to nucleotide positions 123-914 of Seq. ID No.7.

Sequence ID 8: Chimaeric tomato spotted wilt virus S RNA, coding for the coat protein of ToMV and the non-structural protein, NSs in opposite polarity.

Sequence ID 9: The non-structural protein, NSs (in opposite polarity) corresponding to nucleotide positions 1141-2543 of Seq ID No.8.

Examples

All CMV, TSWV, and ToMV RNA-derived sequences presented here are depicted as DNA sequences for the sole purpose of uniformity. It will be appreciated that this is done for convenience only.

Cultivars of *Nicotiana tabacum* and *Lycopersicon esculentum*, used in plant transformation studies, are grown under standard greenhouse conditions. Axenic explant material is grown on standard MS

media [Murashige and Skoog (1962). Physiol. Plant 15:473] containing appropriate phytohormones and sucrose concentrations.

E. coli bacteria are grown on rotary shakers at 37°C in standard LB-medium. *Agrobacterium tumefaciens* strains are grown at 28°C in MinA medium supplemented with 0.1 % glucose [Ausubel et al., (1987). Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Inter-sciences, New York, Chichester, Brisbane, Toronto, and Singapore].

In all cloning procedures the *E. coli* strain JM83 , (F^-, Δ(*lac-pro*), *ara*, *rps*L, Ø80, d*lac*ZM15) is used as the preferred recipient for recombinant plasmids.

Binary vectors are conjugated to *Agrobacterium tumefaciens* strain LBA 4404, a strain containing the Ti-plasmid *vir* region, [Hoekema et al (1983). Nature 303: 179] in standard triparental matings using the *E. coli* HB101, containing the plasmid pRK2013 as a helper strain. [Figurski and Helinski, (1979). Proc. Natl. Acad. Sci. USA 76: 1648]. Appropriate *Agrobacterium tumefaciens* recipients are selected on media containing rifampicin (50 µg/ml) and kanamycine (50 µg/ml).

Cloning of fragments in the vectors pUC19 [Yanish-Perron et al (1985). Gene 33: 103], pBluescript (Stratagene), pBIN19 [Bevan et al (1984). Nucl. Acids Res. 12: 8711] or derivatives, restriction enzyme analysis of DNA, transformation to *E. coli* recipient strains, isolation of plasmid DNA on small as well as large scale, nick-translation, *in vitro* transcription, DNA sequencing, Southern blotting and DNA gel electrophoresis are performed according to standard procedures [Maniatis et al (1982).

Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, New York; Ausubel et al supra, (1987)].

DNA amplification using the polymerase chain reaction (PCR) was performed as recommended by the supplier of the *Taq* polymerase (Perkin Elmer Cetus). Amplification of RNA by reverse transcription and subsequent standard DNA amplification was performed using the Gene Amp RNA PCR as recommended by the supplier (Perkin Elmer Cetus).

Example 1: Isolation of CMV particles and genetic material therein

A CMV serogroup I is isolated from squash and maintained on squash by mechanical passaging. Virus is purified from systemically infected squash leaves essentially following the procedure according to Francki et al [(1979) CMI/AAB Descr. of Plant Viruses 213]. Approximately 100 µg of virus in a volume of 250 µl is extracted with phenol, then with a mixture of phenol and chloroform and finally with chloroform. RNA is precipitated with ethanol and collected by centrifugation . The pellet is dissolved in 20 µl of water.

Example 2: Isolation of ToMV particles and genetic material therein

A ToMV isolate from tomato, is maintained on tobacco via mechanical passaging. Virus is purified from systemically infected tobacco leaves essentially following the method essentailly according to Hollings & Huttinga [(1976) CMI/AAB Descr. of Plant Viruses 156]. Approximately 200 µg of virus in a volume of 300 µl is extracted with phenol, then with a mixture of phenol and chloroform and finally with chloroform. The RNA is precipitated with ethanol and collected by centrifugation. The pellet is dissolved in 50 µl of water.

Example 3: Molecular cloning of CMV RNA 3

The sequence of RNA 3 of CMV is isolated using RNA-based PCR on purified CMV RNA (Perkin Elmer Cetus supra). Two primers are designed, ZUP069:

(5' TTTGGATCCA CGTGGTCTCC TTTTGGAG 3'),

which is complementary to the first 16 nucleotides at the 3' end of RNA 3 of CMV (Seq. Id No.1), and ZUP068 :

(5' TTTGGATCCG TAATCTTACC ACT 3')

which is identical to the first 14 nucleotides at the 5' end of RNA 3 of CMV (Seq. Id. No.1). Both primers contain BamH1 restriction sites to enable further cloning of the amplified DNA molecules. Purified CMV RNA is subjected to the Gene Amp RNA PCR, and the resulting PCR fragment is isolated from an agarose gel and cloned into Sma1-linearized pUC19, yielding the recombinant plasmid pZU181.

Example 4: Molecular cloning of TSWV S RNA

A cDNA clone containing almost the complete TSWV S RNA-specific sequence was constructed by fusion of cDNA clones 520 and 614 on the unique EcoR1 site yielding pTSWV-S1 [De Haan et al (1990). J. Gen. Virol. 71: 1001]. The complete sequence of TSWV S RNA is isolated using RNA-based PCR on purified pTSWV-S1 DNA (Perkin Elmer Cetus supra). Two primers are designed, ZUP250:

5'(TTTGGATCCA GAGCAATCGT GTCAATTTTG TGTTCATACC TTAAC) 3'

which comprises 36 nucleotides identical to the first 36 nucleotides at the 5' end of TSWV S RNA (Seq. Id. No.8), and ZUP251:

5'(TTTGGATCCA GAGCAATTGT GTCAGAATTT TGTTCATAAT CAAACCTCAC TT) 3'

which comprises 43 nucleotides complimentary to the first 43 nucleotides at the 3' end of TSWV S RNA (Seq. Id. No.8). Both primers contain BamH1 restriction sites to enable further cloning of the amplified DNA molecules. The resulting PCR fragment is isolated from an agarose gel and cloned into Sma1-linearized pUC19, yielding the recombinant plasmid pTSWV-S2.

Example 5: Molecular cloning of the CP and P30 genes of ToMV

The sequence of the genes corresponding to the coat protein (CP) and P30 of ToMV is isolated using RNA-based PCR. Primer ZUP112 spans either side of the translational start codon of the CP gene of ToMV RNA:

5' GTATTAACCA TGGCTTACTC 3' (comprising 13 nucleotides identical to nucleotides 121-133 of Seq. Id. No.1) and

primer ZUP113 spans either side of the translational stop codon of the CP gene of ToMV RNA:

5' GCACCCATGG ATTTAAGATG 3' (comprising 16 nucleotides complementary to nucleotides 595-610 of Seq. Id. No.1), and

primer ZUP117 spans either side of the translational start codon of the P30 gene of ToMV RNA:

5' TATTTCTCCA TGGCTCTAGT 3' (comprising 13 nucleotides identical to nucleotides 121-133 of Seq. Id No.6,) and

primer ZUP118 spans either side of the translational stop codon of the P30 gene of ToMV RNA:

5' GAGTAAGCCA TGGTTAATAC 3' (comprising 13 nucleotides complementary to nucleotides 911-923 of Seq. Id. No.6)

The primers contain Nco1 restriction sites to enable further cloning of the amplified DNA molecules. Purified ToMV RNA is subjected to the Gene Amp RNA PCR. Resulting PCR fragments are isolated from an agarose gel and cloned into Sma1-linearized pUC19, yielding the recombinant plasmids pZU183 (containing the CP gene) and pZU206 (containing the P30 gene).

Example 6: Synthesis of the ribonuclease T1 gene

The sequence of the gene corresponding with ribonuclease T1 is synthesized on a commercial DNA synthesizer (Pharmacia LKB, Gene assembler plus) as primer ZUP110 (comprising nucleotides identical to nucleotides 121-293 of Seq. Id No.4):

5' TTTCCATGGC ATGCGACTAC ACTTGCGGTT CTAACTGCTA CTCTTCTTCA
GACGTTTCTA CTGCTCAAGC TGCCGGATAT AAACTTCACG AAGACGGTGA
AACTGTTGGA TCTAATTCTT ACCCACACAA ATACAACAAC TACGAAGGTT
TTGATTTCTC TGTGAGCTCT CCCTAC 3'

and primer ZUP111 (comprising nucleotides complementary to nucleotides 278-446 of Seq. Id. No.4):

5' GGGCCATGGT TATGTACATT CAACGAAGTT GTTACCAGAA GCACCAGTGT
GAGTGATAAC ACCAGCATGT TGGTTGTTTT CGTTGAAGAC GACACGGTCA
GCACCTGGAG AAGGACCAGA GTAAACATCA CCGCTAGAGA GGATAGGCCA
TTCGTAGTAG GGAGAGCTCA C 3'

Both primers contain NcoI restriction sites to enable further cloning of the amplified DNA molecules. The primers are annealed and subjected to a standard DNA PCR. The amplified DNA fragment is isolated from an agarose gel and cloned into SmaI-linearized pUC19, yielding the recombinant plasmid pZU230.

Example 7: Construction of an expression vector pZU-A

The 35S cauliflower mosaic virus (CaMV) promoter fragment is isolated from the recombinant plasmid pZO27, a derivative of pUC19 carrying as a 444 bp HindIII-PstI fragment the HincII-HphI region of the 35S promoter of CaMV strain Cabb-S [Franck et al (1980). Cell 21: 285-294]. The nucleotide sequences of CaMV strains are very similar for the different strains . The 35S promoter fragment is excised from pZO27 as a 472 bp EcoRI-PstI fragment which contains: a part of the polylinker region,

437 bp of the non-transcribed region and the transcription initiation site and 7 bp of the non-translated leader region but not containing any 35S translational initiators. The 35S promoter fragment is ligated using T4 ligase into EcoRI-PstI linearized pZO008. The plasmid pZO008 carries the nopaline synthase (NOS) polyadenylation signal as a 270 bp PstI-HindIII fragment. The resulting recombinant plasmid pZU-A carries the 35S promoter, a unique PstI site and the NOS terminator [Gielen et al (1991) Bio/Technology 10:1363].

Example 8: Construction of a plant transformation vector, which yields a transcript which replicates upon infection with CMV.

The 5' end of the minus-sense RNA 3 of CMV is fused directly to the transcription initiation site of the CaMV 35S promoter using two primers ZUP148:

5' CCACGTCTTC AAAGCAAG 3' (complementary to nucleotides of the CaMV 35S promoter), and primer ZUP146:

5'CTTCGCACCT TCGTGGGGGC TCCAAAAGGA GACCACCTCT CCAAATGAAA 3' (comprising nucleotides complementary to nucleotides 1860-1827 of Seq. Id. No.1)

with pZU-A as a template in a standard DNA PCR reaction. The amplified DNA fragment is digested with EcoRV and cloned in EcoRV linearized pZU-A. The resulting plasmid is digested with BstX1 and Pst1 and purified on an agarose gel. pZU181 is digested with Pst1 and BstX1, the 2.1 kb insert DNA is purified on an agarose gel and subsequently cloned into the gel-purified pZU-A derivative, yielding pCMV3AS-1.

The movement protein (MP) coding domain of pCMV3AS-1 is replaced by a unique Ncol cloning site and the axehead structure of the *Hepatitis* delta viral RNA is cloned downstream of the 3' end of the minus-sense RNA 3 of CMV, by PCR amplification of two DNA fragments using pCMV3AS-1 as a

template. The first DNA fragment is amplified using primers ZUP050:

5' AGCTGCTAAC GTCTTATTAA G 3' (comprising nucleotides complementary to nucleotides 1020-1039 of Seq. Id. No.1)

and ZUP329:

5' GTCTTTAGCA CCATGGTG 3' (comprising nucleotides identical to nucleotides 604-612 of Seq Id. No.1)

The DNA fragment is digested with Nru1 and Nco1 and a 411 bp long DNA fragment (position 607-1016 Seq. Id. No.1) is isolated from an agarose gel. The second DNA fragment is amplified using primers ZUP327:

5' GGAGAGCCAT GGCTCGGG 3' (comprising nucleotides complementary to nucleotides 115-126 of Seq. Id. No.1)

and ZUP350, a primer synthesised with nucleotides comprising nucleotides complementary to antigenomic hepatitis delta virus RNA as described by Perrotta AT & Been MD (1991) Nature Vol 350(4) pp434-436 ligated to nucleotides identical to nucleotides 1-14 (3' end of the primer) of Seq. Id. No. 1:

5'TTTCTGCAGA TCTTAGCCAT CCGAGTGGA CGTGCGTCCT CCTTCGGATG CCCAGGTCGG ACCGCGAGGA GGTGGAGATG CCATGCCGAC CCGTAATCTT ACCACT)3'.

The DNA fragment is digested with Pst1 and Nco1 and a 208 bp. long DNA fragment is isolated from an agarose gel. Both isolated DNA fragments are cloned in pCMV3AS-1, linearized with Pst1 and Nru1, to yield pCMV3AS-2. Genes coding for elicitors (example 5) or cell inhibitory proteins (example 6) can be cloned as Nco1 DNA fragments into the unique Nco1 site of pCMV3AS-2. The resulting pCMV3AS-2 derived plasmids are digested with HindIII and the DNA fragments containing the

chimaeric genes are isolated from an agarose gel and ligated into HindIII linearized pBIN19, resulting in binary plant transformation vectors pBINCMV3-CP, pBINCMV3-P30 and pBINCMV3-T1 respectively.

Example 9: Construction of a plant transformation vector, which yields a transcript which replicates upon infection with TSWV.

The 5' end of the minus-sense TSWV S RNA is fused directly to the transcription initiation site of the CaMV 35S promoter using two primers ZUP148 (Example 8), and primer ZUP255:

5' ACACAATTGC TCTCCTCTCC AAATGAAA 3' (comprising nucleotides identical to nucleotides 2608-2621 of Seq. Id. No.8)

with pZU-A as a template in a standard DNA PCR reaction. The amplified DNA fragment is digested with EcoRV and cloned in EcoR5 linearized pZU-A. The resulting plasmid is digested with Mun1 and Pst1 and purified on an agarose gel. pTSWV-S2 is digested with Pst1 and Mun1, the 2.9 kb insert DNA is purified on an agarose gel and subsequently cloned into the gel-purified pZU-A derivative, yielding pTSWVSAS-1.

The N coding domain of pTSWVSAS-1 is replaced by a unique Nco1 cloning site and the axehead structure of the *Hepatitis* delta viral RNA is cloned downstream of the 3' end of the minus-sense TSWV S RNA, by PCR amplification of two DNA fragments using pTSWVSAS-1 as a template. The first DNA fragment is amplified using primers ZUP252:

5' GACCCGAAAG GGACCAATTT C 3' (comprising nucleotides complimentary to nucleotides 911-930 of Seq Id. No.8)

and ZUP253:

5' TTTCCATGGC TGTAAGTTAA ATT 3' (comprising nucleotides identical to nucleotides 636-655 of Seq Id. No.8)

The DNA fragment is digested with Ball and Ncol and a 269 bp long DNA fragment (position 636-911 Sequence Id No.8) is isolated from an agarose gel. The second DNA fragment is amplified using primers ZUP254:

5' TTTCCATGGT GATCGTAAAA G 3' (comprising nucleotides complementary to nucleotides 140-157 of Seq. Id No.8)

and ZUP255 a primer synthesised with nucleotides comprising nucleotides complementary to antigenomic hepatitis delta virus RNA as described by Perrotta AT & Been MD (1991) Nature Vol 350(4) pp434-436, ligated to nucleotides identical to nucleotides 1-14 (3' end of the primer) of Seq. Id. No. 8:

5' TTTCTGCAGA TCTTAGCCAT CCGAGTGGAC GTGCGTCCTC CTTCGGATGC CCAGGTCGGA CCGCGAGGAG GTGGAGATGC CATGCCGACC CAGAGCAATC GTGTC 3'

The DNA fragment is digested with Pstl and Ncol and a 245 bp. long DNA fragment is isolated from an agarose gel. Both isolated DNA fragments are cloned in pTSWVSAS-1, linearized with Pstl and Ball, to yield pTSWVSAS-2. Genes coding for elicitors (example 5) or cell inhibitory proteins (example 6) are cloned as Ncol DNA fragments into the unique Ncol site of pTSWVSAS-2. The resulting pTSWVSAS-2 derived plasmids are digested with Xbal and the DNA fragments containing the chimaeric genes are isolated from an agarose gel and ligated into Xbal linearized pBIN19, resulting in binary plant transformation vectors pBINTSWVS-CP (Seq Id No.8), pBINTSWVS-P30 and pBINTSWVS-T1 respectively.

Example 10: Selection of suitable host plants

1) Tobacco, *Nicotiana tabacum* var. Samsun EN. A tobacco cultivar harboring the N' gene of *N. sylvestris* showing an HS response upon infection with ToMV. The CP of ToMV elicits a strong HSR defense reaction in this host.

2) Tomato, *Lycopersicon esculentum* var. ATV847, parental line for commercial hybrids Yaiza and Gemma. A tomato line harboring the Tm-2^2 resistance gene to ToMV. It has been demonstrated that the P30 of ToMV elicits a HS response in this resistag30 nt genotype [Fraser (1986) CRC Crit. Rev. Plant Sci.3: 257; Keen (1990). Ann. Rev. Genet. 24: 447].

Example 11: Transformation of binary vectors to tobacco and tomato plant material

Methods to transfer binary vectors to plant material are well established and known to a person skilled in the art. Variations in procedures exist due to for instance differences in used *Agrobacterium* strains, different sources of explant material, differences in regeneration systems depending on as well the cultivar as the plant species used.

The binary plant transformation vectors as described above are used in plant transformation experiments according to the following procedures. Binary vector constructs are transferred by tri-parental mating to an acceptor *Agrobacterium tumefaciens* strain, followed by southern analysis of the ex-conjugants for verification of proper transfer of the construct to the acceptor strain, inoculation and cocultivation of axenic explant material with the *Agrobacterium tumefaciens* strain of choice, selective killing of the *Agrobacterium tumefaciens* strain used with appropriate antibiotics, selection of transformed cells by growing on selective media containing kanamycine, transfer of tissue to shoot-inducing media, transfer of selected shoots to root inducing media, transfer of plantlets to soil, assaying for intactness of the construct by southern analyses of isolated total DNA from the transgenic plant, assaying for proper function of the inserted chimaeric gene by northern analysis and/or enzyme assays and western blot analysis of proteins [Ausubel et al supra, (1987)].

Example 12: Expression of chimaeric sequences in tobacco and tomato plant cells

RNA is extracted from leaves of regenerated plants using the following protocol. Grind 200 mg leaf material to a fine powder in liquid nitrogen. Add 800 µl RNA extraction buffer (100 mM Tris-HCl (pH 8,0), 500 mM NaCl, 2 mM EDTA, 200 mM β-Mercapto-ethanol, 0,4% SDS) and extract the homogenate with phenol, collect the nucleic acids by alcohol precipitation. Re suspend the nucleic acids in 0,5 ml 10 mM Tris-HCl (pH 8,0), 1 mM EDTA, add LiCl to a final concentration of 2 M, leave on ice for maximally 4 hours and collect the RNA by centrifugation. Re suspend in 400 µl 10 mM Tris-HCl (pH 8,0), 1 mM EDTA and precipitate with alcohol, finally re-suspend in 50 µl 10 mM Tris-HCl (pH 8,0), 1 mM EDTA. RNAs are separated on glyoxal/agarose gels and blotted to Genescreen as described by van Grinsven et al [(1986). Theor. Appl. Gen. 73:94-101]. Recombinant viral RNA sequences are detected using DNA or RNA probes labeled with [^{32}P], [^{35}S] or by using non-radioactive labeling techniques. Based on northern analysis, it is determined to what extent the regenerated plants express the chimaeric recombinant viral genes.

Plants transformed with recombinant viral DNA sequences are also subjected to western blot analysis after inoculation with the respective virus. Proteins are extracted from leaves of transformed plants by grinding in sample buffer according to Laemmli [(1970). Nature 244: 29]. A 50 µg portion of protein is subjected to electrophoresis in a 12,5 % SDS-polyacrylamide gel essentially as described by Laemmli supra, (1970). Separated proteins are transferred to nitrocellulose electrophoretically as described by Towbin et al [(1979). Proc. Natl. Acad. Sci. USA 76: 4350]. Transferred proteins are reacted with antiserum raised against purified ToMV particles or against purified P30 protein, according to Towbin et al supra, (1979). Based on the results of the western analysis,

it is determined that transformed plants do express elicitor proteins after inoculation with the respective virus.

Example 13: Resistance of tobacco and tomato plants against CMV or TSWV infection.

Transformed plants are grown in the greenhouse under standard quarantine conditions in order to prevent any infections by pathogens. The transformants are self-pollinated and the seeds harvested. Progeny plants are analyzed for segregation of the inserted gene and subsequently infected with CMV or TSWV by mechanical inoculation. Tissue from plants systemically infected with CMV or TSWV is ground in 5 volumes of ice-cold inoculation buffer (10 mM phosphate buffer) and rubbed in the presence of carborundum powder on the first two fully extended leafs of approximately 5 weeks old seedlings. Inoculated plants are monitored for symptom development during 3 weeks after inoculation.

Plants containing CMV Related DNA Sequences or TSWV related DNA sequences show reduced susceptibility to CMV or TSWV infection compared with untransformed control plants which show severe systemic CMV or TSWV symptoms within 7 days after inoculation.

SEQUENCE LISTING

Sequence ID No.1

Sequence type: Nucleotide
Sequence length: 1860 nucleotides
Strandness: Single stranded
Molecule type: Chimaeric cucumber mosaic virus RNA 3 coding for the CP of ToMV.

GTAATCTTAC CACTCGTGTG TGTGCGTGTG TGTGTGTCGA GTCGTGTTGT CCGCACATTT 60

GAGTCGTGCT GTCCGCACAT ATATTTTACC TTTTGTGTAC AGTGTGTTAG ATTTCCCGAG 120

CCATGGCTTA CTCAATCACT TCTCCATCGC AATTTGTGTT TTTGTCATCT GTATGGGCTG 180

ACCCTATAGA ATTGTTAAAC GTTTGTACAA ATTCGTTAGG TAACCAGTTT CAAACACAGC 240

AAGCAAGAAC TACTGTTCAA CAGCAGTTCA GCGAGGTGTG GAAACCTTTC CCTCAGAGCA 300

CCGTCAGATT TCCTGGCGAT GTTTATAAGG TGTACAGGTA CAATGCAGTT TTAGATCCTC 360

TAATTACTGC GTTGCTGGGG TCTTTCGATA CTAGGAATAG AATAATCGAA GTAGAAAACC 420

AGCAGAATCC GACAACAGCT GAAACGTTAG ATGCTACCCG CAGGGTAGAC GACGCTACGG 480

TTGCAATTCG GTCTGCTATA AATAATTTAG TTAATGAACT AGTAAGAGGT ACTGGACTGT 540

ACAATCAAAA TACTTTTGAA AGTATGTCTG GGTTGGTCTG GACCTCTGCA CCTGCATCTT 600

AAATCCATGG TGTATTAGTA TATAAGTATT GTGAGTCTGT ACATAATACT ATATCTATAG 660

TGTCCTGTGT GAGTTGATAC AGTAGACATC TGTGACGCGA TGCCGTGTTG AGAAGGGAAC 720

ACATCTGGTT TTAGTAAGCC TACATCACAG TTTTGAGGTT CAATTCCTCA TACTCCCTGT 780

TGAGTCCCTT ACTTTCTCAT GGATGCTTCT CCGCGAGATT GCGTTATTGT CTACTGACTA 840

TATAGAGAGT GTGTGTGCTG TGTTTTCTCT TTTGTGTCGT AGAATTGAGT CGAGTCATGG 900

ACAAATCTGA ATCAACCAGT GCTGGTCGTA ACCGTCGACG TCGTCCGCGT CGTGGTTCCC 960

GCTCCGCCCC CTCCTCCGCG GATGCTAACT TTAGAGTCTT GTCGCAGCAG CTTTCGCGAC 1020

TTAATAAGAC GTTAGCAGCT GGTCGTCCAA CTATTAACCA CCCAACCTTT GTAGGGAGTG 1080

AACGCTGTAA ACCTGGGTAC ACGTTCACAT CTATTACCCT AAAGCCACCA AAAATAGACC 1140

GTGGGTCTTA TTACGGTAAA AGGTTGTTAT TACCTGATTC AGTCACGGAA TATGATAAGA 1200

AACTTGTTTC GCGCATTCAA ATTCGAGTTA ATCCTTTGCC GAAATTCGAT TCTACCGTGT 1260

GGGTGACAGT CCGTAAAGTT CCTGCCTCCT CGGACTTATC CGTTGCCGCC ATCTCTGCTA 1320

TGTTTGCGGA CGCCGCATTT GGAGTCCAAG CTAACAACAA ATTGTTGTAT GATCTTTCGG 1380

CGGGAGCCTC ACCGGTACTG GTTTATCAGT ACATGCGCGC TGATATAGGT GACATGAGAA 1440

AGTACGCCGT CCTCGTGTAT TCAAAAGACG ATGCGCTCGA GACGGACGAG CTAGTACTTC 1500

ATGTTGACAT CGAGCACCAA CGCATTCCCA CATCTAGAGT ACTCCCAGTC TGATTCCGTG 1560

TTCCCAGAAC CCTCCCTCCG ATTTCTGTGG CGGGAGCTGA GTTGGCAGTT CTGCTATAAA 1620

CTGTCTGAAG TCACTAAACG TTTTACGGTG AACGGGTTGT CCATCCAGCT TACGGCTAAA 1680

ATGGTCAGTC GTGGAGAAAT CCACGCCAGC AGATTTACAA ATCTCTGAGG CGCCTTTGAA 1740

ACCATCTCCT AGGTTTTTTC GGAAGGACTT CGGTCCGTGT ACCTCTAGCA CAACGTGCTA 1800

GTCTTAGGGT ACGGGTGCCC CTTGTCTTCG CACCTTCGTG GGGGCTCCAA AAGGAGACCA 1860

Sequence ID No.2

Sequence type: Amino acid
Sequence length: 159 amino acids
Strandness: Single stranded
Molecule type: Coat protein of ToMV (corresponding to nucleotides from positions 123-599 of Seq. ID. No.1).

Met Ala Tyr Ser Ile Thr Ser Pro Ser Gln Phe Val Phe Leu Ser 15

Ser Val Trp Ala Asp Pro Ile Glu Leu Leu Asn Val Cys Thr Asn 30

Ser Leu Gly Asn Gln Phe Gln Thr Gln Gln Ala Arg Thr Thr Val 45

Gln Gln Gln Phe Ser Glu Val Trp Lys Pro Phe Pro Gln Ser Thr 60

Val Arg Phe Pro Gly Asp Val Tyr Lys Val Tyr Arg Tyr Asn Ala 75

Val Leu Asp Pro Leu Ile Thr Ala Leu Leu Gly Ser Phe Asp Thr 90

Arg Asn Arg Ile Ile Glu Val Glu Asn Gln Gln Asn Pro Thr Thr 105

Ala Glu Thr Leu Asp Ala Thr Arg Arg Val Asp Asp Ala Thr Val 120

Ala Ile Arg Ser Ala Ile Asn Asn Leu Val Asn Glu Leu Val Arg 135

Gly Thr Gly Leu Tyr Asn Gln Asn Thr Phe Glu Ser Met Ser Gly 150

Leu Val Trp Thr Ser Ala Pro Ala Ser 159

Sequence ID No.3

Sequence type: Amino acid
Sequence length: 218 amino acids
Strandness: Single stranded
Molecule type: **Coat protein of cucumber mosaic virus corresponding to nucleotide positions from 897-1550 of Seq. ID. No 1.**

Met Asp Lys Ser Glu Ser Thr Ser Ala Gly Arg Asn Arg Arg Arg 15

Arg Pro Arg Arg Gly Ser Arg Ser Ala Pro Ser Ser Ala Asp Ala 30

Asn Phe Arg Val Leu Ser Gln Gln Leu Ser Arg Leu Asn Lys Thr 45

Leu Ala Ala Gly Arg Pro Thr Ile Asn His Pro Thr Phe Val Gly 60

Ser Glu Arg Cys Lys Pro Gly Tyr Thr Phe Thr Ser Ile Thr Leu 75

Lys Pro Pro Lys Ile Asp Arg Gly Ser Tyr Tyr Gly Lys Arg Leu 90

Leu Leu Pro Asp Ser Val Thr Glu Tyr Asp Lys Lys Leu Val Ser 105

Arg Ile Gln Ile Arg Val Asn Pro Leu Pro Lys Phe Asp Ser Thr 120

Val Trp Val Thr Val Arg Lys Val Pro Ala Ser Ser Asp Leu Ser 135

Val Ala Ala Ile Ser Ala Met Phe Ala Asp Gly Ala Ser Pro Val 150

Leu Val Tyr Gln Tyr Ala Ala Phe Gly Val Gln Ala Asn Asn Lys 165

Leu Leu Tyr Asp Leu Ser Ala Met Arg Ala Asp Ile Gly Asp Met 180

Arg Lys Tyr Ala Val Leu Val Tyr Ser Lys Asp Asp Ala Leu Glu 195

Thr Asp Glu Leu Val Leu His Val Asp Ile Glu His Gln Arg Ile 210

Pro Thr Ser Arg Val Leu Pro Val 218

Sequence ID No.4

Sequence type: Nucleotide

Sequence length: 1696 nucleotides
Strandness: Single stranded
Molecule type: Chimaeric cucumber mosaic virus RNA 3 coding for RNAse T1.

GTAATCTTAC CACTCGTGTG TGTGCGTGTG TGTGTGTCGA GTCGTGTTGT CCGCACATTT 60

GAGTCGTGCT GTCCGCACAT ATATTTTACC TTTTGTGTAC AGTGTGTTAG ATTTCCCGAG 120

CCATGGCATG CGACTACACT TGCGGTTCTA ACTGCTACTC TTCTTCAGAC GTTTCTACT 180

CTCAAGCTGC CGGATATAAA CTTCACGAAG ACGGTGAAAC TGTTGGATCT AATTCTTACC 240

CACACAAATA CAACAACTAC GAAGGTTTTG ATTTCTCTGT GAGCTCTCCC TACTACGAAT 300

GGCCTATCCT CTCTAGCGGT GATGTTTACT CTGGTGGTTC TCCAGGTGCT GACCGTGTCG 360

TCTTCAACGA AAACAACCAA CTAGCTGGTG TTATCACTCA CACTGGTGCT TCTGGTAACA 420

ACTTCGTTGA ATGTACATAA CCATGGTGTA TTAGTATATA AGTATTGTGA GTCTGTACAT 480

AATACTATAT CTATAGTGTC CTGTGTGAGT TGATACAGTA GACATCTGTG ACGCGATGCC 540

GTGTTGAGAA GGGAACACAT CTGGTTTTAG TAAGCCTACA TCACAGTTTT GAGGTTCAAT 600

TCCTCATACT CCCTGTTGAG TCCCTTACTT TCTCATGGAT GCTTCTCCGC GAGATTGCGT 660

TATTGTCTAC TGACTATATA GAGAGTGTGT GTGCTGTGTT TTCTCTTTTG TGTCGTAGAA 720

TTGAGTCGAG TCATGGACAA ATCTGAATCA ACCAGTGCTG GTCGTAACCG TCGACGTCGT 780

CCGCGTCGTG GTTCCCGCTC CGCCCCCTCC TCCGCGGATG CTAACTTTAG AGTCTTGTCG 840

CAGCAGCTTT CGCGACTTAA TAAGACGTTA GCAGCTGGTC GTCCAACTAT TAACCACCCA 900

ACCTTTGTAG GGAGTGAACG CTGTAAACCT GGGTACACGT TCACATCTAT TACCCTAAAG 960

CCACCAAAAA TAGACCGTGG GTCTTATTAC GGTAAAAGGT TGTTATTACC TGATTCAGTC 1020

ACGGAATATG ATAAGAAACT TGTTTCGCGC ATTCAAATTC GAGTTAATCC TTTGCCGAAA 1080

TTCGATTCTA CCGTGTGGGT GACAGTCCGT AAAGTTCCTG CCTCCTCGGA CTTATCCGTT 1140

GCCGCCATCT CTGCTATGTT TGCGGACGGA GCCTCACCGG TACTGGTTTA TCAGTACGCC 1200

GCATTTGGAG TCCAAGCTAA CAACAAATTG TTGTATGATC TTTCGGCGAT GCGCGCTGAT 1260

ATAGGTGACA TGAGAAAGTA CGCCGTCCTC GTGTATTCAA AAGACGATGC GCTCGAGACG 1320

GACGAGCTAG TACTTCATGT TGACATCGAG CACCAACGCA TTCCCACATC TAGAGTACTC 1380

CCAGTCTGAT TCCGTGTTCC CAGAACCCTC CCTCCGATTT CTGTGGCGGG AGCTGAGTTG 1440

GCAGTTCTGC TATAAACTGT CTGAAGTCAC TAAACGTTTT ACGGTGAACG GGTTGTCCAT 1500

CCAGCTTACG GCTAAAATGG TCAGTCGTGG AGAAATCCAC GCCAGCAGAT TTACAAATCT 1560

CTGAGGCGCC TTTGAAACCA TCTCCTAGGT TTTTTCGGAA GGACTTCGGT CCGTGTACCT 1620

CTAGCACAAC GTGCTAGTCT TAGGGTACGG GTGCCCCTTG TCTTCGCACC TTCGTGGGGG 1680

CTCCAAAAGG AGACCA 1696

Sequence ID No. 5

Sequence type: Amino Acid
Sequence length: 105 amino acids
Strandness: Single stranded
Molecule type: RNAse T1 corresponding to positions 123-437 of Seq. Id No. 4.

Met Ala Cys Asp Tyr Thr Cys Gly Ser Asn Cys Tyr Ser Ser Ser 15

Asp Val Ser Thr Ala Gln Ala Ala Gly Tyr Lys Leu His Glu Asp 30

Gly Glu Thr Val Gly Ser Asn Ser Tyr Pro His Lys Tyr Asn Asn 45

Tyr Glu Gly Phe Asp Phe Ser Val Ser Ser Pro Tyr Tyr Glu Trp 60

Pro Ile Leu Ser Ser Gly Asp Val Tyr Ser Gly Gly Ser Pro Gly 75

Ala Asp Arg Val Val Phe Asn Glu Asn Asn Gln Leu Ala Gly Val 90

Ile Thr His Thr Gly Ala Ser Gly Asn Asn Phe Val Glu Cys Thr 105

Sequence ID No.6
Sequence type: Nucleotide
Sequence length: 2173 nucleotides
Strandness: Single stranded
Molecule type: Chimaeric cucumber mosaic virus RNA 3, coding for P30 of ToMV.

GTAATCTTAC CACTCGTGTG TGTGCGTGTG TGTGTGTCGA GTCGTGTTGT CCGCACATTT 60

GAGTCGTGCT GTCCGCACAT ATATTTTACC TTTTGTGTAC AGTGTGTTAG ATTTCCCGAG 120

CCATGGCTCT AGTTGTTAAA GGTAAGGTAA ATATTAATGA GTTTATCGAT CTGTCAAAGT 180

CTGAGAAACT TCTCCCGTCG ATGTTCACGC CTGTAAAGAG TGTTATGGTT TCAAAGGTTG 240

ATAAGATTAT GGTCCATGAA AATGAATCAT TGTCTGAAGT AAATCTCTTA AAAGGTGTAA 300

AACTTATAGA AGGTGGGTAT GTTTGCTTAG TTGGTCTTGT TGTGTCCGGT GAGTGGAATT 360

TCCCAGATAA TCGCCGTGGT GGTGTGAGTG TCTGCATGGT TGACAAGAGA ATGGAAAGAG 420

CGGACGAAGC CACACTGGGG TCATATTACA CTGCTGCTGC TAAAAAGCGG TTTCAGTTTA 480

AAGTGGTCCC AAATTACGGT ATTACAACAA AGGATGCAGA AAAGAACATA TGGCAGGTCT 540

TAGTAAATAT TAAAAATGTA AAAATGAGTG CGGGCTACTG CCCTTTGTCA TTAGAATTTG 600

TGTCTGTGTG TATTGTTTAT AAAAATAATA TAAAATTGGG TTTGAGGGAG AAAGTAACGA 660

GTGTGAACGA TGGAGGACCC ATGGAACTTT CGGAAGAAGT TGTTGATGAG TTCATGGAGA 720

ATGTTCCAAT GTCGGTTAGA CTCGCAAAGT TTCGAACCAA ATCCTCAAAA AGAGGTCCGA 780

AAAATAATAA TAATTTAGGT AAGGGGCGTT CAGGCGGAAG GCCTAAACCA AAAAGTTTTG 840

ATGAAGTTGA AAAAGAGTTT GATAATTTGA TTGAAGATGA AGCCGAGACG TCGGTCGCGG 900

ATTCTGATTC GTATTAACCA TGGTGTATTA GTATATAAGT ATTGTGAGTC TGTACATAAT 960

ACTATATCTA TAGTGTCCTG TGTGAGTTGA TACAGTAGAC ATCTGTGACG CGATGCCGTG 1020

TTGAGAAGGG AACACATCTG GTTTTAGTAA GCCTACATCA CAGTTTTGAG GTTCAATTCC 1080

TCATACTCCC TGTTGAGTCC CTTACTTTCT CATGGATGCT TCTCCGCGAG ATTGCGTTAT 1140

TGTCTACTGA CTATATAGAG AGTGTGTGTG CTGTGTTTTC TCTTTTGTGT CGTAGAATTG 1200

AGTCGAGTCA TGGACAAATC TGAATCAACC AGTGCTGGTC GTAACCGTCG ACGTCGTCCG 1260

CGTCGTGGTT CCCGCTCCGC CCCCTCCTCC GCGGATGCTA ACTTTAGAGT CTTGTCGCAG 1320

CAGCTTTCGC GACTTAATAA GACGTTAGCA GCTGGTCGTC CAACTATTAA CCACCCAACC 1380

TTTGTAGGGA GTGAACGCTG TAAACCTGGG TACACGTTCA CATCTATTAC CCTAAAGCCA 1440

CCAAAAATAG ACCGTGGGTC TTATTACGGT AAAAGGTTGT TATTACCTGA TTCAGTCACG 1500

GAATATGATA AGAAACTTGT TTCGCGCATT CAAATTCGAG TTAATCCTTT GCCGAAATTC 1560

GATTCTACCG TGTGGGTGAC AGTCCGTAAA GTTCCTGCCT CCTCGGACTT ATCCGTTGCC 1620

GCCATCTCTG CTATGTTTGC GGACGCCGCA TTTGGAGTCC AAGCTAACAA CAAATTGTTG 1680

TATGATCTTT CGGCGGGAGC CTCACCGGTA CTGGTTTATC AGTACATGCG CGCTGATATA 1740

GGTGACATGA GAAAGTACGC CGTCCTCGTG TATTCAAAAG ACGATGCGCT CGAGACGGAC 1800

GAGCTAGTAC TTCATGTTGA CATCGAGCAC CAACGCATTC CCACATCTAG AGTACTCCCA 1860

GTCTGATTCC GTGTTCCCAG AACCCTCCCT CCGATTTCTG TGGCGGGAGC TGAGTTGGCA 1920

GTTCTGCTAT AAACTGTCTG AAGTCACTAA ACGTTTTACG GTGAACGGGT TGTCCATCCA 1980

GCTTACGGCT AAAATGGTCA GTCGTGGAGA AATCCACGCC AGCAGATTTA CAAATCTCTG 2040

AGGCGCCTTT GAAACCATCT CCTAGGTTTT TTCGGAAGGA CTTCGGTCCG TGTACCTCTA 2100

GCACAACGTG CTAGTCTTAG GGTACGGGTG CCCCTTGTCT TCGCACCTTC GTGGGGGCTC 2160

CAAAAGGAGA CCA 2173

Sequence ID No.7
Sequence type: Amino acid
Sequence length: 264 amino acids
Strandness: Single stranded
Molecule type: P30 of ToMV corresponding to nucleotide positions 123-914 of Seq. ID No.6.

Met Ala Leu Val Val Lys Gly Lys Val Asn Ile Asn Glu Phe Ile 15

Asp Leu Ser Lys Ser Glu Lys Leu Leu Pro Ser Met Phe Thr Pro 30

Val Lys Ser Val Met Val Ser Lys Val Asp Lys Ile Met Val His 45

Glu Asn Glu Ser Leu Ser Glu Val Asn Leu Leu Lys Gly Val Lys 60

Leu Ile Glu Gly Gly Tyr Val Cys Leu Val Gly Leu Val Val Ser 75

Gly Glu Trp Asn Phe Pro Asp Asn Arg Arg Gly Gly Val Ser Val 90

Cys Met Val Asp Lys Arg Met Glu Arg Ala Asp Glu Ala Thr Leu 105

Gly Ser Tyr Tyr Thr Ala Ala Ala Lys Lys Arg Phe Gln Phe Lys 120

Val Val Pro Asn Tyr Gly Ile Thr Thr Lys Asp Ala Glu Lys Asn 135

Ile Trp Gln Val Leu Val Asn Ile Lys Asn Val Lys Met Ser Ala 150

Gly Tyr Cys Pro Leu Ser Leu Glu Phe Val Ser Val Cys Ile Val 165

Tyr Lys Asn Asn Ile Lys Leu Gly Leu Arg Glu Lys Val Thr Ser 180

Val Asn Asp Gly Gly Pro Met Glu Leu Ser Glu Glu Val Val Asp 195

Glu Phe Met Glu Asn Val Pro Met Ser Val Arg Leu Ala Lys Phe 210

Arg Thr Lys Ser Ser Lys Arg Gly Pro Lys Asn Asn Asn Asn Leu 225

Gly Lys Gly Arg Ser Gly Gly Arg Pro Lys Pro Lys Ser Phe Asp 240

Glu Val Glu Lys Glu Phe Asp Asn Leu Ile Glu Asp Glu Ala Glu 255

Thr Ser Val Ala Asp Ser Asp Ser Tyr 264

Sequence ID No.8

Sequence type: Nucleotide
Sequence length: 2621 nucleotides
Strandness: Single stranded
Molecule type: Chimaeric tomato spotted wilt virus S RNA, coding for the coat protein of ToMV and the non-structural protein, NSs in opposite polarity.

AGAGCAATCG TGTCAATTTT GTGTTCATAC CTTAACACTC AGTCTTACAA ATCATCACAT 60

TAAGAACCTA AGAAACGACT GCGGGATACA GAGTTGCACT TTCGCACCTT GAGTTACATA 120

CGGTCAAAGC ATATAACAAC TTTTACGATC ACCATGGCTT ACTCAATCAC TTCTCCATCG 180

CAATTTGTGT TTTTGTCATC TGTATGGGCT GACCCTATAG AATTGTTAAA CGTTTGTACA 240

AATTCGTTAG GTAACCAGTT TCAAACACAG CAAGCAAGAA CTACTGTTCA ACAGCAGTTC 300

AGCGAGGTGT GGAAACCTTT CCCTCAGAGC ACCGTCAGAT TTCCTGGCGA TGTTTATAAG 360

GTGTACAGGT ACAATGCAGT TTTAGATCCT CTAATTACTG CGTTGCTGGG GTCTTTCGAT 420

ACTAGGAATA GAATAATCGA AGTAGAAAAC CAGCAGAATC CGACAACAGC TGAAACGTTA 480

GATGCTACCC GCAGGGTAGA CGACGCTACG GTTGCAATTC GGTCTGCTAT AAATAATTTA 540

GTTAATGAAC TAGTAAGAGG TACTGGACTG TACAATCAAA ATACTTTTGA AAGTATGTCT 600

GGGTTGGTCT GGACCTCTGC ACCTGCATCT TAAATCCATG GCTGTAAGTT AAATTATAAA 660

AAAGCCTATA AATATATAAA GCTTTCTTTA TCTTTATTGC TTGTGCTTGC TTAGTGTGTT 720

AAATTTTAAA TAAGTGTGTT TAATTAAAGT TTGCTTTCTG TGTGTTGTGC TTAATAAATA 780

ATAAAATAAC AAAAACAACG AAAACAAAAA ATAAATAAAA TAAAAATAAA ATAAAAATAA 840

AATAAAATAA AAATAAAATA AAAAATAAAA AACAAAAAAC AAAAAACAAA AACAAAACCC 900

AAATTTGGCC AAATTGGTCC CTTTCGGGTC TTTTTGGTTT TTCGTTTTTT AATTTTTTGT 960

TGTTTTTATT TCATTTTTTG ATTTTATTTT ATTTTAATTT TATTTTCATT TTTATTTTTT 1020

GTTTTTATGG TTTCTACTAG ACAGGAGGAA TTTGAAAGAG ATGACAAACA GAGAAATAAT 1080

TATAAGTAAA GAAAGAAAAT AAACATAACA TAATTAGAAA AAGCTGGACA AAGCAAGATT 1140

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AATCAAGCTT TAACAAAGAT TTTGCAACTG AAATAGATTG TGGAGAAATT TTAATTTCTC 1260

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ATGTGTGAGT TTCGAGCCAG AGCATTGGAA CCGATCCTTT GGGGTATGAA GGGTCATGAA 1560

CAATGTTGTA AGGCTCCTTT AAATCAGAAA ACATCATTGA TAATTCAAAA GGAGCTTTGC 1620

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TGTTATGGTT TGGAATGCAG GCAATAGATA AATAAAATGT TTTGTTTGTT TCATCTCCTG 1740

CAACCTTGAA CAATTTCTGA ATGGAAACCT GCTTCAAAAC CTTTGGAACC CTTAGCCAGA 1800

GGCTCAGCTT GAAATGAGAA TCAGTGGAAG CTTGAGAGTT AGGCATGATG TTGTTTTCTG 1860

CTGACATGAG CAGAGATTTC ACTGCAAGAG AATTTACAGT TCTGTTGTTG CTTTCAACTT 1920

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TTTCTTGGAG CTGGAATCGG TCTGTAATAT CAGATCTGTT CATGATAGAT TCAATAGAGT 2160

GGAGCTGGGC AGGAGACAAA ACCTTCAAAT GACCTTGATG TTTCACTCCG TTAGCATTGA 2220

CTGTATTTGA GCAAACAGAT AGTGCCAGAA CAGAGTTATC AATATTGATG CTAAAATCAA 2280

TATCATCAAA AATAGGGATA TACACATGCT GAGAAAGAAA TCTCTTCTTC TTCACAGGGA 2340

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CTTTACCAGA TGCAGTTGAT CCCCAGACTG AAGCTCTTGT CTGAATGATC GACTCATAAA 2520

CACTTGAAGA CATTATGGTT ATTGGTACTG TGTTCTTATT ACAGTATTGT GATTTTCTAA 2580

GTGAGGTTTG ATTATGAACA AAATTCTGAC ACAATTGCTC T 2621

Sequence ID No.9

Sequence type: Amino acid
Sequence length: 464 amino acids
Strandness: Single stranded
Molecule type: The non-structural Protein, NSs corresponding to nucleotide positions 1142-2533 (in opposite polarity) of Seq ID No.8.

Met Ser Ser Ser Val Tyr Glu Ser Ile Ile Gln Thr Arg Ala Ser 15

Val Trp Gly Ser Thr Ala Ser Gly Lys Ala Val Val Asp Ser Tyr 30

Trp Ile His Glu Leu Gly Thr Gly Ser Gln Leu Val Gln Thr Gln 45

Leu Tyr Ser Asp Ser Arg Ser Lys Val Val Leu Trp Leu Tyr Cys 60

Lys Val Gly Ile Phe Pro Val Lys Lys Lys Arg Phe Leu Ser Gln 75

His Val Tyr Ile Pro Ile Phe Asp Asp Ile Asp Phe Ser Ile Asn 90

Ile Asp Asn Ser Val Leu Ala Leu Ser Val Cys Ser Asn Thr Val 105

Asn Ala Asn Gly Val Lys His Gln Gly His Leu Lys Val Leu Ser 120

Pro Ala Gln Leu His Ser Ile Glu Ser Ile Met Asn Arg Ser Asp 135

Ile Thr Asp Arg Phe Gln Leu Gln Glu Lys Asp Ile Ile Pro Asn 150

Asp Lys Tyr Ile Glu Ala Ala Asn Lys Gly Ser Leu Ser Cys Val 165

Lys Glu His Thr Tyr Lys Ile Glu Met Cys Tyr Asn Gln Ala Leu 180

Gly Lys Val Asn Val Leu Ser Pro Asn Arg Asn Val His Glu Trp 195

Leu Tyr Ser Phe Lys Pro Asn Phe Asn Gln Val Glu Ser Asn Asn 210

Arg Thr Val Asn Ser Leu Ala Val Lys Ser Leu Leu Met Ser Ala 225

Glu Asn Asn Ile Met Pro Asn Ser Gln Ala Ser Thr Asp Ser His 240

Phe Lys Leu Ser Leu Trp Leu Arg Val Pro Lys Val Leu Lys Gln 255

Val Ser Ile Gln Lys Leu Phe Lys Val Ala Gly Asp Glu Thr Asn 270

Lys Thr Phe Tyr Leu Ser Ile Ala Cys Ile Pro Asn His Asn Ser 285

Val Glu Thr Ala Leu Asn Ile Thr Val Ile Cys Lys His Gln Leu 300

Pro Ile Arg Lys Cys Lys Ala Pro Phe Glu Leu Ser Met Met Phe 315

Ser Asp Leu Lys Glu Pro Tyr Asn Ile Val His Asp Pro Ser Tyr 330

Pro Lys Gly Ser Val Pro Met Leu Trp Leu Glu Thr His Thr Ser 345

Leu His Lys Phe Phe Ala Thr Asn Leu Gln Glu Asp Val Ile Ile 360

Tyr Thr Leu Asn Asn Leu Glu Leu Thr Pro Gly Lys Leu Asp Leu 375

Gly Glu Arg Thr Leu Asn Tyr Ser Glu Asp Ala Tyr Lys Arg Lys 390

Tyr Phe Leu Ser Lys Thr Leu Glu Cys Leu Pro Ser Asn Thr Gln 405

Thr Met Ser Tyr Leu Asp Ser Ile Gln Ile Pro Ser Trp Lys Ile 420

Asp Phe Ala Arg Gly Glu Ile Lys Ile Ser Pro Gln Ser Ile Ser 435

Val Ala Lys Ser Leu Leu Lys Leu Asp Leu Ser Gly Ile Lys Lys 450

Lys Glu Ser Lys Val Lys Glu Ala Tyr Ala Ser Gly Ser Lys 464

CLAIMS

1. A DNA construct capable of encoding directly or indirectly for a minus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by a virus when invading a plant such that at least one eliciting element is produced as a consequence of the interaction with the RNA dependent RNA polymerase encoded by the said invading virus, which construct is under expression control of a promoter and a terminator capable of functioning in plants.

2. A recombinant DNA construct capable of encoding for a plus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by a virus when invading a plant and producing as a result of such interaction a plus sense RNA molecule which is capable of encoding for at least one eliciting element.

3. A construct according to claim 1 comprising Seq. Id. No. 1

4. A construct according to claim 1 comprising Seq. Id. No.4.

5. A construct according to claim 1 comprising Seq. Id. No.6.

6. A construct according to claim 1 comprising Seq. Id. No.8.

7. A recombinant DNA construct according to claim 1 or claim 2 wherein the eliciting element is selected from the group comprising proteins, polypeptides or peptides.

8. A construct according to Claim 7 wherein the eliciting element is capable of eliciting a hypersensitive response or the release of a cell inhibitory protein in a plant.

9. A construct according to Claim 1 or Claim 2 wherein the elicitor protein is of plant virus, bacterial, fungal or

nematode origin.

10. A construct according to Claim 1 or Claim 2 wherein the cell inhibitory protein is selected from the group comprising ribonucleases, proteinases, ribosomal inhibitory proteins, and cell wall degrading proteins.

11. A construct according to any one of Claims 1 to 10 wherein the construct comprises a constitutive promoter selected from the group consisting of viral, fungal, bacterial and plant derived promoters.

12. A construct according to Claim 11 wherein the promoter is selected from the group consisting of CaMV 19S, nopaline synthase, octopine synthase, heat shock 80 promoters.

13. Plants containing in their genome a construct according to any one of Claims 1 to 12.

14. A process of preparing plants according to Claim 13, which comprises:

A) Inserting into the genome of a plant cell a DNA construct according to Claim 1;

B) obtaining transformed cells;

C) regenerating from the transformed cells genetically transformed plants.

Figure 1.

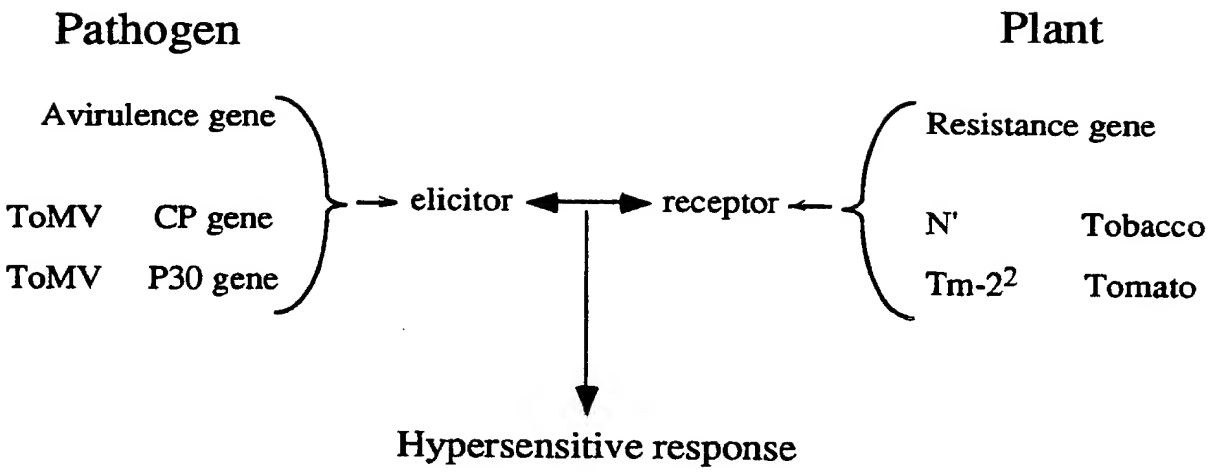

Pathogen
Plant
Avirulence gene
ToMV CP gene
ToMV P30 gene
elicitor
receptor
Resistance gene
N' Tobacco
Tm-2² Tomato
Hypersensitive response

Figure 2.

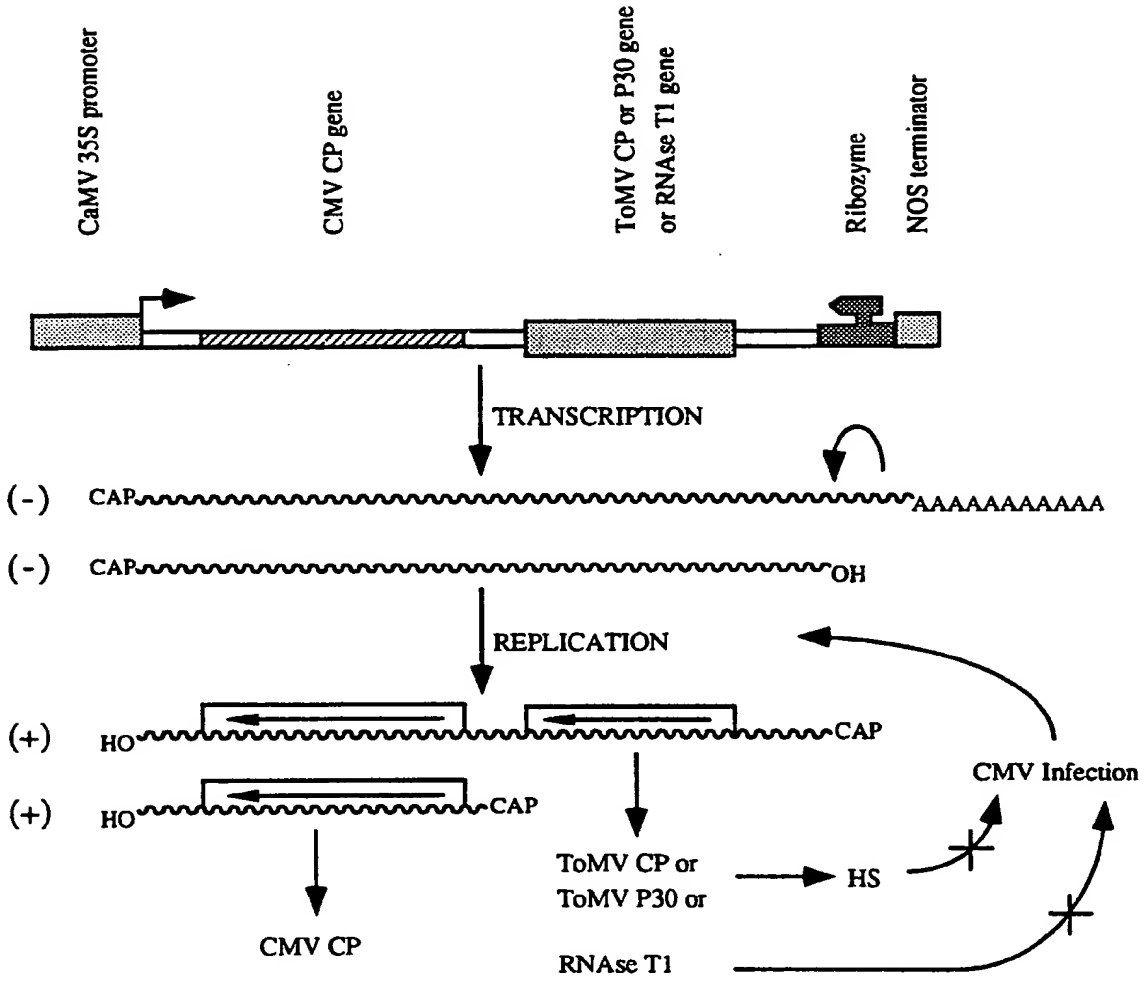
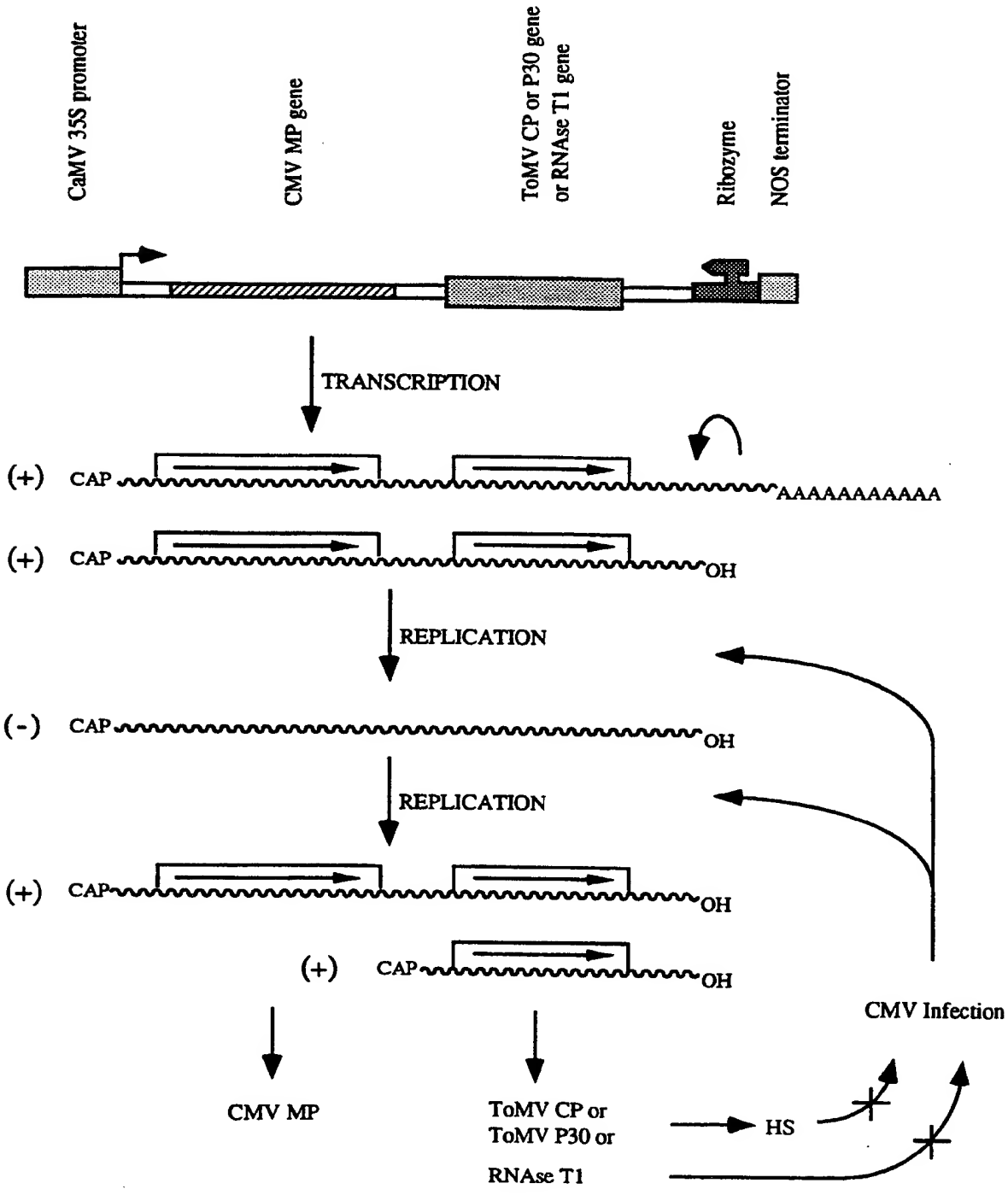


Figure 3



INTERNATIONAL SEARCH REPORT

Internatior pplication No
PCT/EP 94/01817

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 13994 (CSIRO) 19 September 1991 see page 7, line 33 - page 8, line 10 see page 14, line 30 - line 35 ---	1,7,8, 10-14
X	CHEMICAL ABSTRACTS, vol. 113, no. 15, 1990, Columbus, Ohio, US; abstract no. 127723, JUN, W. 'Preparation of transgenic plants for control of virosis' ---	1,7,8, 10-14
Y	see abstract & CN,A,1 033 645 (CHINESE ACADEMY OF SCIENCES) 5 July 1989 ---	9
Y	EP,A,0 298 918 (CIBA-GEIGY) 11 January 1989 see claim 14 ---	9
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 October 1994	16. 11. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Maddox, A

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INTERNATIONAL SEARCH REPORT

Internation pplication No
PCT/EP 94/01817

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 479 180 (HOECHST) 8 April 1992 see the whole document	1,7,8, 10-14
X	STADLER GENETICS SYMPOSIA SERIES: GENE MANIPULATION IN PLANT IMROVEMENT, II. EDITED BY J. PERRY GUSTAFSON, 1990 pages 313 - 330 YOUNG, M., ET AL. 'Using plant virus and related RNA sequences to control gene expression' see page 318 - page 319	1,7,8, 10-14
X	EP,A,0 425 004 (AVEVE) 2 May 1991 see page 7, line 15 - line 24; example 1	1,14
X	AU,B,7 195 191 (NIHON NOHYAKU) 12 March 1992 see page 24, line 4 - line 6	1,7,9,14
P,X	EP,A,0 573 767 (NIHON NOHYAKU) 15 December 1993 see page 9, line 20 - line 21	1
A	BIOLOGICAL ABSTRACTS, vol. 92 1991, Philadelphia, PA, US; abstract no. 129186, CULVER, J.N., ET AL. 'Tobacco mosaic virus elicitor coat protein genes produce a hypersensitive phenotype in transgenic Nicotiana sylvestris' see abstract & MOL. PLANT MICROBE INTERACT., vol.4, no.5, 1991 pages 458 - 463	9
A	BIOLOGICAL ABSTRACTS, vol. 94 1992, Philadelphia, PA, US; abstract no. 89218, PFITZNER, U.M., ET AL. 'Expression of a viral avirulence gene in transgenic plants is sufficient to induce the hypersensitive defense reaction' see abstract & MOL. PLANT MICOBE INTERACT., vol.5, no.4, 1992 pages 318 - 321	9

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP 94/01817

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9113994	19-09-91	NONE		
CN-A-1033645		NONE		
EP-A-0298918	11-01-89	AU-B-	620039	13-02-92
		JP-A-	1037294	07-02-89
EP-A-0479180	08-04-92	CA-A-	2052808	06-04-92
		JP-A-	5260971	12-10-93
EP-A-0425004	02-05-91	NL-A-	8902452	01-05-91
		NL-A-	9001711	01-05-91
		CA-A-	2026703	04-04-91
		JP-A-	3280883	11-12-91
AU-B-7195191		NONE		
EP-A-0573767	15-12-93	AU-B-	3824893	04-11-93
		JP-A-	6046874	22-02-94